JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2004, 55, 3, 551–561
www.jpp.krakow.pl

R. RUKKUMANI, K. ARUNA, P. SURESH VARMA, VP. MENON*

INFLUENCE OF FERULIC ACID ON CIRCULATORY PROOXIDANT - ANTIOXIDANT STATUS DURING ALCOHOL AND PUFA INDUCED TOXICITY

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India.

In recent years, there has been an escalation in alcohol abuse and inevitably, alcohol related disorders are becoming an increasingly important cause of morbidity and mortality. Alcohol is known to induce a dose dependent increase in lipid peroxidation. Alcohol related disabilities are more pronounced when taken along with diet rich in polyunsaturated fatty acid (PUFA). The present work aims at analysing the protective role of ferulic acid (FA), a naturally occurring nutritional component on alcohol and PUFA induced oxidative stress. Two different doses of ferulic acid, 20 mg/kg body weight and 40 mg /kg body weight were used for the study. The results showed that the levels of oxidative markers; thiobarbituric acid reactive substances (TBARS), hydroperoxides (HP) and levels of copper (Cu) and ferritin were increased significantly in plasma of alcohol, thermally oxidised PUFA (ΔPUFA) and alcohol + ΔPUFA groups, which were decreased significantly on treatment with both the doses of ferulic acid. The activities of enzymic antioxidants viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X) and non enzymic antioxidants like vitamin C, vitamin E, and reduced glutathione (GSH) and the levels of zinc (Zn) were significantly decreased in alcohol, ΔPUFA and alcohol + ΔPUFA groups which were improved significantly on treatment with both the doses of FA. The reduction in oxidative stress was more significant in 20 mg/kg body weight treatment groups compared to 40 mg/kg body weight. Thus from the results obtained, we conclude that FA effectively protects the system against alcohol and PUFA induced oxidative stress.

Key words: *Alcohol, PUFA, Ferulic acid, Lipid peroxidation, Antioxidants, Oxidative stress, Copper, Zinc, Ferritin.*

INTRODUCTION

Chronic alcohol ingestion is associated with a variety of pathological conditions varying from simple intoxication to severe, life threatening derangement of metabolism (1). Increasing evidence demonstrates that oxidative stress plays an important etiologic role in the development of alcohol related disorders (2,3). Intake of alcohol results in excessive generation of free radicals (4), which alter the biomembranes and cause severe damage.

Fat is an important dietary component, which affects both growth and health. In recent years there has been increased focus on replacing some of the individual fat intake with unsaturated fat. Current data indicate that newer heart friendly oils like sunflower oil possess a high PUFA n-6 content and high n-6/n-3 ratio, which are actually detrimental to health (5). Moreover heating of oil is known to alter its nutritional properties especially when it is rich in PUFA. During deep fat frying many volatile and nonvolatile products are produced, some of which are toxic depending on the level of intake (6).

Alcoholics usually after a heavy binge of alcohol, take fried food items normally made up of PUFA. The combined ingestion of alcohol and PUFA is dangerous and results in pathological conditions (7).

In the present study, we have analysed the impact of ferulic acid - commonly found in fruits and vegetables such as tomato, sweet corn and rice bran [8] on alcohol and PUFA induced oxidative stress. Ferulic acid has been known to be effective against cancer, cold, flu, skin aging, muscle wasting, influenza etc (Nutra pack report). Since little or no work has been done on the *in vivo* antioxidant potential of ferulic acid, we were interested in investigating its influence over alcohol and PUFA induced oxidative stress in male Wistar rats.

MATERIALS AND METHODS

Animals

Male Albino rats, Wistar strain of body weight ranging 140-160 g bred in Central Animal House, Rajah Muthiah Medical College, Tamil Nadu, India, fed on pellet diet (Agro Corporation Private Limited, Bangalore, India) were used for the study and water was given *ad libitum*. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrates). It provides metabolisable energy of 3600 K Cal/Kg.

The animals were housed in plastic cages under controlled conditions of 12h light / 12 h dark cycle, 50% humidity and at 30° ± 2°C. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the Animal Ethical Committee, Annamalai University.

Materials used

Ethanol	: Absolute ethanol [AR] was obtained from Hayman Limited, England.
Thermally Oxidised PUFA	: Sunflower oil was subjected to heating at 180°C for 30 minutes, twice.
Ferulic Acid	: Ferulic acid was obtained from Sigma Aldrich Private Limited, Bangalore, India.

Experimental design

The animals were divided into 12 groups of 6 rats each.

Group 1 (Control)	: Control rats were given standard pellet diet and glucose solution isocalorific to ethanol and high fat diet.
Group 2 (Alcohol)	: Rats given 20% ethanol [7.9 g/kg body weight] [9] orally using an intragastric tube.
Group 3 (ΔPUFA)	: Rats given high fat diet [15% heated sunflower oil] [7] mixed with the diet.
Group 4 (Alcohol + ΔPUFA)	: Rats given 20% ethanol + 15% heated sunflower oil [7].
Group 5 (Alcohol + FA [1])	: Rats given Ferulic acid [40 mg/kg body weight] dissolved in 20% ethanol.
Group 6 (Alcohol + FA [2])	: Rats given Ferulic acid [20 mg/kg body weight] dissolved in 20% ethanol.
Group 7 (ΔPUFA + FA [1])	: Rats given 15% heated sunflower oil + Ferulic acid [40 mg/kg body weight] dissolved in water.
Group 8 (ΔPUFA + FA [2])	: Rats given 15% heated sunflower oil + Ferulic acid [20 mg/kg body weight] dissolved in water.
Group 9 (Al + ΔP + FA [1])	: Rats given 20% ethanol + 15% heated sunflower oil + Ferulic acid [40 mg/kg body weight].
Group 10 (Alc + ΔP + FA [2])	: Rats given 20% ethanol + 15% heated sunflower oil + Ferulic acid [20 mg/kg body weight].
Group 11 (FA [1])	: Rats given ferulic acid [40 mg/kg body weight] dissolved in water orally using an intragastric tube.
Group 12 (FA [2])	: Rats given ferulic acid [20 mg/kg body weight] dissolved in water orally using an intragastric tube.

All rats were maintained on isocalorific diet using glucose solution. (Total calories per day: 508 K Cal/150 g rat).

At the end of the experimental period of 45 days, the rats were sacrificed and the blood was collected in heparinised tubes and plasma was separated for various biochemical estimations.

Preparation of hemolysate

After separation of plasma, the packed cells were washed thrice with physiological saline. A known volume of erythrocyte was lysed with hypotonic phosphate buffer, pH 7.4. The hemolysate was separated by centrifugation at 2500 x g for 15 min at 2°C.

Biochemical investigation

Lipid peroxidation as evidenced by the formation of TBARS and HP were measured by the method of Niehaus and Samuelsson (10) and Jiang *et al.* (11) respectively. The antioxidant status

was assessed by analysing the levels of GSH by Ellman's method (12), vitamin E by Baker and Frank (13), vitamin C by Roe and Kuether method (14). The activities of enzymic antioxidants viz, SOD by Kakkar *et al.* (15), CAT by the method of Sinha (16), and GP_X by the method of Ellman's were also analysed. The levels of copper and zinc were determined by atomic absorption spectrometer (Aanalyst 800, USA) (17) and ferritin was analysed by ELISA (18).

RESULTS

Table 1 shows the levels of lipid peroxidative indices in plasma. The levels of TBARS and HP were increased significantly in alcohol, ΔPUFA and alcohol + ΔPUFA groups, which were decreased significantly on treatment with both the doses of ferulic acid. The decrease was more significant in 20 mg/kg body weight treated groups compared to 40 mg/kg body weight. The levels of non enzymic antioxidants: vitamin C, vitamin E and GSH (*Table 2*) and enzymic antioxidants: SOD, CAT and GP_X (*Table 3*) were significantly depleted in alcohol, ΔPUFA and alcohol + ΔPUFA groups which were improved significantly on treatment with ferulic acid. The improvement was more significant in 20 mg/kg body weight FA treated groups.

The levels of copper and ferritin were significantly elevated and of zinc were significantly decreased (*Table 4*) in alcohol, ΔPUFA and alcohol + ΔPUFA groups which were positively modulated by both the doses of ferulic acid. But 20 mg/kg body weight treatment was found to be more effective compared to 40 mg/kg

Table 1. Levels of Lipid peroxidative indices in plasma
(Values are mean ± S.D from 6 rats in each group)

S. No.	Groups	TBARS mM/dl	Hydroperoxides $\times 10^{-5}$mM/dl
1.	Control	0.125 ± 0.01^{akl}	7.467 ± 0.36^{ahkl}
2.	Alcohol	0.363 ± 0.02^{b}	20.000 ± 2.37^{bci}
3.	ΔPUFA	0.332 ± 0.02^{c}	19.583 ± 1.74^{cbi}
4.	Alcohol + ΔPUFA	0.445 ± 0.07^{d}	30.333 ± 2.80^{d}
5.	Alcohol + FA (1)	0.208 ± 0.02^{eg}	15.417 ± 1.24^{egj}
6.	Alcohol + FA (2)	0.167 ± 0.01^{fh}	10.250 ± 0.94^{fh}
7.	ΔPUFA + FA (1)	0.203 ± 0.02^{ge}	14.250 ± 0.94^{gej}
8.	ΔPUFA + FA (2)	0.163 ± 0.01^{hf}	8.958 ± 0.71^{hafkl}
9.	Alcohol + ΔPUFA + FA (1)	0.280 ± 0.01^{i}	19.750 ± 1.54^{ibc}
10.	Alcohol + ΔPUFA + FA (2)	0.245 ± 0.02^{j}	15.333 ± 1.08^{jeg}
11.	FA (1)	0.125 ± 0.01^{kal}	7.483 ± 0.56^{kahl}
12.	FA (2)	0.125 ± 0.01^{lak}	7.467 ± 0.33^{lahk}

ANOVA followed by DMRT
Values not sharing the common superscript differ significantly at $P \leq 0.05$

Table 2. Levels of non-enzymic antioxidants in plasma
(Values are mean ± S.D from 6 rats in each group)

S. No. Groups	Vitamin C mg/dl	Vitamin E mg/dl	GSH mg/dl
1. Control	1.880 ± 0.09^{akl}	1.672 ± 0.09^{akl}	39.333 ± 3.39^{akl}
2. Alcohol	1.150 ± 0.07^{b}	0.633 ± 0.03^{bc}	17.917 ± 1.28^{bdi}
3. ΔPUFA	1.292 ± 0.06^{ci}	0.685 ± 0.03^{eb}	23.083 ± 2.20^{cej}
4. Alcohol + ΔPUFA	0.611 ± 0.03^{d}	0.378 ± 0.04^{d}	15.333 ± 1.87^{db}
5. Alcohol + FA (1)	1.488 ± 0.06^{egj}	1.025 ± 0.06^{egj}	25.167 ± 2.64^{ecgj}
6. Alcohol + FA (2)	1.625 ± 0.08^{fgh}	1.400 ± 0.09^{fh}	32.417 ± 3.67^{fh}
7. ΔPUFA + FA (1)	1.552 ± 0.08^{gefj}	1.083 ± 0.08^{gej}	27.667 ± 2.94^{gej}
8. ΔPUFA + FA (2)	1.688 ± 0.09^{hf}	1.467 ± 0.06^{hf}	34.167 ± 2.86^{hf}
9. Alcohol + ΔPUFA + FA (1)	1.308 ± 0.14^{ic}	0.873 ± 0.04^{i}	19.500 ± 1.87^{ib}
10. Alcohol + ΔPUFA + FA (2)	1.512 ± 0.04^{jeg}	1.018 ± 0.04^{jeg}	26.000 ± 2.70^{jceg}
11. FA (1)	1.892 ± 0.12^{kal}	1.652 ± 0.09^{kal}	39.167 ± 4.07^{kal}
12. FA (2)	1.895 ± 0.09^{lak}	1.652 ± 0.09^{lak}	41.000 ± 3.85^{lak}

ANOVA followed by DMRT
Values not sharing the common superscript differ significantly at $P \leq 0.05$

Table 3. Activities of enzymic antioxidants in hemolysate
(Values are mean ± S.D from 6 rats in each group)

S. No. Groups	SOD units*/mg Hb	CAT units**/mg Hb	GPx units***/mg Hb
1. Control	4.950 ± 0.36^{akl}	3.967 ± 0.35^{akl}	29.167 ± 2.32^{akl}
2. Alcohol	1.533 ± 0.17^{bci}	1.275 ± 0.12^{b}	15.400 ± 1.05^{bci}
3. ΔPUFA	1.800 ± 0.16^{cbi}	1.567 ± 0.19^{ci}	16.167 ± 0.82^{cbi}
4. Alcohol + ΔPUFA	1.200 ± 0.13^{d}	0.933 ± 0.15^{d}	12.333 ± 1.44^{d}
5. Alcohol + FA (1)	3.408 ± 0.28^{eg}	2.617 ± 0.23^{egj}	19.500 ± 1.87^{egj}
6. Alcohol + FA (2)	4.225 ± 0.37^{fh}	3.283 ± 0.33^{fh}	25.250 ± 2.36^{fh}
7. ΔPUFA + FA (1)	3.617 ± 0.33^{ge}	2.650 ± 0.24^{gej}	20.083 ± 2.49^{gej}
8. ΔPUFA + FA (2)	4.358 ± 0.29^{hf}	3.533 ± 0.27^{hf}	25.750 ± 2.32^{hf}
9. Alcohol + ΔPUFA + FA (1)	1.700 ± 0.14^{ibc}	1.750 ± 0.16^{ic}	16.333 ± 1.40^{ibc}
10. Alcohol + ΔPUFA + FA (2)	2.442 ± 0.34^{j}	2.525 ± 0.25^{jeg}	18.917 ± 1.77^{jeg}
11. FA (1)	4.833 ± 0.38^{kal}	3.983 ± 0.29^{kal}	30.167 ± 2.48^{kal}
12. FA (2)	5.150 ± 0.33^{lak}	4.075 ± 0.25^{lak}	30.667 ± 2.58^{lak}

ANOVA followed by DMRT
Values not sharing the common superscript differ significantly at $P \leq 0.05$
*Enzyme reaction which gives 50% inhibition of NBT reduction/min
**µmole of H_2O_2 liberated/min.
***µmole of glutathione utilised/min.

Table 4. Levels of Ferritin, Zinc and Copper in plasma
(Values are mean ± S.D from 6 rats in each group)

S. No.Groups	Ferritin (ng/ml)	Zinc (µg/dl)	Copper (µg/dl)
1. Control	5.050 ± 0.19^{a}	202.667 ± 2.07^{akl}	121.167 ± 4.02^{akl}
2. Alcohol	14.317 ± 0.12^{b}	102.667 ± 2.88^{b}	289.333 ± 7.53^{bc}
3. ΔPUFA	13.633 ± 0.12^{ci}	107.167 ± 1.47^{c}	285.667 ± 5.96^{cb}
4. Alcohol + ΔPUFA	19.017 ± 0.17^{d}	77.167 ± 1.47^{d}	350.000 ± 4.19^{d}
5. Alcohol + FA (1)	11.317 ± 0.12^{e}	140.500 ± 2.17^{e}	186.500 ± 5.01^{e}
6. Alcohol + FA (2)	7.100 ± 0.09^{f}	171.500 ± 2.26^{f}	167.833 ± 5.53^{f}
7. ΔPUFA + FA (1)	10.517 ± 0.12^{g}	151.500 ± 2.35^{gj}	178.000 ± 5.02^{gj}
8. ΔPUFA + FA (2)	6.767 ± 0.12^{h}	178.833 ± 2.71^{h}	142.33 ± 5.35^{h}
9. Alcohol + ΔPUFA + FA (1)	13.633 ± 0.15^{ic}	115.00 ± 1.89^{i}	240.500 ± 6.41^{i}
10. Alcohol + ΔPUFA + FA (2)	9.567 ± 0.16^{j}	151.000 ± 1.79^{jg}	179.000 ± 4.98^{jg}
11. FA (1)	5.117 ± 0.29^{ka}	203.000 ± 1.67^{kal}	124.500 ± 6.22^{kal}
12. FA (2)	4.983 ± 0.15^{la}	201.833 ± 1.60^{lak}	119.333 ± 9.07^{lak}

ANOVA followed by DMRT
Values not sharing the common superscript differ significantly at $P \leq 0.05$

body weight. In our study, we also tried a lower dosage (10 mg/kg body weight), which was not found to be effective and hence data is not included.

DISCUSSION

It is a well-established fact that alcoholic patients and experimental animals exposed to ethanol display biochemical signs of oxidative damage. Evidences have indicated that free radicals or reactive oxygen species (ROS), such as α-hydroxy ethylradical, superoxide radical, hydroxy radical, peroxy radical and hydrogen peroxide are implicated in ethanol induced oxidative tissue injury (19,20). Ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme in the liver and acetaldehyde is oxidised to acetate by aldehyde oxidase or xanthine oxidase giving rise to ROS via cyt P450 2E1 (21,22). Thus excess generation of ROS as a consequence of induction of cytochrome P450 2E1 by ethanol consumption plays a major role in the development of lipid peroxidation (23) and formation of lipid peroxidative products and hence in our study, there was a significant elevation in TBARS and HP in alcohol treated group.

The changes in the composition of erythrocyte with increased erythrocyte deformability *ex vivo* have been reported with increased intake of PUFA (24). The increase in dietary unsaturated fat increases the degree of unsaturation of the membranes (25) and unsaturated bonds are more susceptible to lipid

peroxidation. Moreover heating of oil rich in PUFA produces various toxic metabolites (26), which may increase the lipid peroxidative changes. Thus, in our study, the levels of TBARS and HP were higher in ΔPUFA treated group.

The combined ingestion of alcohol and ΔPUFA resulted in marked elevation in lipid peroxidative indices in our study. Reports have shown that intake of PUFA along with alcohol results in several fold increase in the induction of CYP2E1 (27), which increases the ROS formation and thus lipid peroxidative end products.

There appears to be an inverse correlation between alcohol and PUFA induced lipid peroxidation and antioxidant status. Oxidative stress in the cells refers to an enhanced generation of ROS and/or depletion in antioxidant defense system causing an imbalance between prooxidants and antioxidants. ROS generated in tissues are normally scavenged by enzymatic and non-enzymatic antioxidants.

Antioxidant defense system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites. Vitamin E, the major lipophilic antioxidant and vitamin C, play a vital role in the defense against oxidative stress (28). Glutathione, an important cellular reductant is involved in protection against free radicals, peroxides and other toxic compounds (29). GP_X has a well established role in protecting cells against oxidative stress. Catalase, which acts as preventative antioxidant and SOD, a chain breaking antioxidant, play an important role in protection against the deleterious effects of lipid peroxidation (30). The ingestion of alcohol and PUFA actually challenges the antioxidant defenses by increasing the susceptibility of tissues to free radical induced damage. The decrease in antioxidant status in alcohol, ΔPUFA and alcohol + ΔPUFA groups in our study may be due to their increased utilization to counteract the lipid peroxidative end products.

The micro nutrient cofactors such as Zinc (Zn), Copper (Cu) and Iron (Fe) also play a vital role in maintaining the oxidative status of a cell.

Zinc, an antioxidant acts as a cellular growth protector and its deficiency has been reported in several stages of alcohol intoxication (31). Zn is a critical mineral, involved in different enzyme systems. These include alcohol dehydrogenase (ADH) an enzyme required to detoxify alcohol in the liver. Alcoholics generally have low levels of Zn. This may be due to an inadequate diet or due to alcohol decreasing Zn absorption. This may be a compound problem since low Zn levels decrease the body's ability to metabolize alcohol, resulting in prolonged exposure to high alcohol levels and increased tissue damage (31). The decrease in the levels of Zn in alcohol and PUFA treatment may also be due to the increased utilization of this cofactor by ADH and other antioxidant enzymes.

Several studies have shown that plasma ferritin concentration is increased during alcohol ingestion (32,33). The consumption of excessive amounts of alcohol affects human iron homeostasis and associations of iron overload and heavy alcohol consumption has been recognised for many years (34). Reports suggest that unregulated increase in iron may be due to increased iron absorption via non-carrier

mediated para cellular route during alcohol ingestion (33). The increased ferritin level during alcohol, ΔPUFA and alcohol + ΔPUFA ingestion in our study suggests that, these factors disrupt the normal iron metabolism, resulting in excessive deposition of Fe in liver leading to extensive lipid peroxidation (LPO).

Increased plasma copper concentrations have been observed during chronic ethanol ingestion (35). Cu is transported through plasma with the help of a carrier ceruloplasmin. The increased level of Cu may result in increased level of ceruloplasmin, which is a ferroxidase enzyme. Cu and ceruloplasmin play an important role in the conversion of ferric to ferrous. This redox reaction can enhance the levels of lipid peroxides in circulation. Thus the observed levels of LPO during alcohol and PUFA ingestion can be correlated to the increased levels of Cu in these groups.

Administration of ferulic acid decreased the LPO, improved the antioxidant status and positively modulated the levels of copper, zinc and ferritin. This is mainly because of the antioxidant sparing action of ferulic acid.

The use of antioxidants has been recognised as an important counter measure against condition in which oxidative stress is implicated. Among many classes of compounds, naturally occurring phenolics have been given attention [36]. Explicitly ferulic acid has been shown to possess some activity towards peroxy nitrite [37] and oxidized LDL *in vitro* [38].

Normally phenolic compounds act by scavenging free radicals [39] and quenching the lipid peroxidative chain. The hydroxy and phenoxy groups of phenolic compounds donate their electron to the free radicals and quench them. The phenolic radical in turn forms a quinone methide intermediate, which is excreted *via* the bile [40]. Thus FA being a phenolic compound might have inhibited lipid peroxidation in our study.

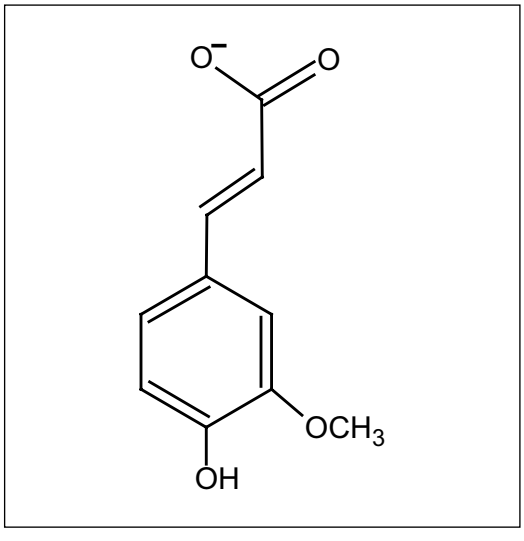

Fig. 1. Ferullic acid (FA)

As seen in *Fig. 1*, FA possesses distinct structural motifs that can possibly contribute to the free radical scavenging capability of this compound. The presence of electron donating groups on the benzene ring [3-methoxy and more importantly 4-hydroxyl] of FA gives additional property for terminating free radical chain reaction. The next functionality - the carboxylic acid group in ferulic acid with adjacent unsaturated C-C double bond can provide additional attack sites for free radicals and thus prevent them from attacking the membrane. In addition, the carboxylic acid group also acts as an anchor of ferulic acid by which it binds to the lipid bilayer providing some protection against lipid peroxidation. Clearly, the presence of electron donating substitutents enhances the antioxidant properties of ferulic acid [41].

Thus ferulic acid, effectively quenches the free radicals, prevents the oxidative stress implemented during alcohol and PUFA induced toxicity.

Although it has been recognised that the use of antioxidant is an important preventive method to minimize the pathological and toxic effects associated with oxidative stress, there are several key considerations that need to be addressed in evaluating a potential antioxidant. The foremost issue deals with the inherent toxicity of the target compound. In our present study, all the three doses did not produce any damage to the system. The lower dosage 10 mg/kg body weight was not effective, because its concentration might not be enough to quench all free radicals generated. The high dose (40 mg/kg body weight) was not so effective compared to 20 mg/kg body weight, because at higher concentration FA might react with some ligands in the system and thus might not be completely available for quenching free radicals.

Second property of an antioxidant is that it should be efficient, protecting at low concentration, preferably capable of inhibiting lipid peroxidation process, where most of the oxidative damage occurs. As presented here, ferulic acid is proved to be a good antioxidant against alcohol and PUFA induced toxicity at a comparatively lower dosage 20 mg/kg body weight.

Thus from the results obtained in this work, we could conclude that ferulic acid, a naturally occurring nutritional compound is a promising candidate as an antioxidant against oxidative stress. Further consideration should be given for making ferulic acid a target, an initial structure for developing a potent drug for the treatment of oxidative stress induced toxicity.

Acknowledgement: Financial support rendered by Jawaharlal Nehru Memorial Fund, New Delhi, India, for the above study is gratefully acknowledged.

REFERENCES

1. Lindi C, Montorfano G, Marciani P. Rat erythrocyte susceptibility to lipid peroxidation after chronic ethanol intake. *Alc* 1998; 16: 311-316.
2. Nordman R. Alcohol and antioxidant system. *Alc Alc* 1994; 29: 818-819.

3. Wieland P, Lauterburg B. Oxidation of mitochondrial proteins and DNA following administration of ethanol. *Biochem Biophys Res Commun* 1995; 24: 815-819.
4. Rouach H, Fataccioli V, Gentil M, French S, Morimoto M, Nordman R. Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. *Hepatology* 1997; 25: 351-355.
5. Sircar S, Kansra V. Choice of cooking oils - Myths and realities. *J Ind Med Assoc* 1998; 96: 304-307.
6. Alexander JC. Chemical and biological properties related to toxicity of heated fats. *J Toxicol Environ Health* 1981; 7: 125-138.
7. Aruna K, Kalpana C, Vishwanathan P, Menon VP. Toxic effects of sunflower oil on ethanol fed rats. *Hepatol Res* 2002; 24: 125-135.
8. Paganga G. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radic Res* 1999; 30: 153-162.
9. Rajakrishnan V, Vishwanathan P, Menon VP. Hepatotoxic effect of alcohol on female rats and siblings: effects of *n*-acetyl cysteine. *Hepatol Res* 1997; 9: 37-50.
10. Niehaus WG, Samuelsson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1968; 6: 126-130.
11. Jiang ZY, Hunt JY, Wolff SP. Detection of lipid hydroperoxides using the 'fox method'. *Anal Biochem* 1992; 202: 384-389.
12. Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
13. Baker H, Frank O, De Angelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Res Int* 1980; 21: 531-536.
14. Roe KM, Kuether CA. Detection of ascorbic acid in whole blood and urine through 2, 4 DNPH derivative of dehydro ascorbic acid. *J Biol Chem* 1943; 147: 399-407.
15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase (SOD). *Ind J Biochem Biophys* 1984; 21: 130-132.
16. Sinha KA. Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389-394.
17. Taylor A, Bryant TN. Comparison of procedures for determination of copper and zinc in serum by atomic absorption spectroscopy. *Clin Chim Acta* 1981; 110: 83-90.
18. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Eng J Med* 1974; 290: 1213-1216.
19. Albano E, Parola M, Comoglio A, Dianzani MU. Evidence for the covalent binding of hydroxyethyl radicals to rat liver microsomal proteins. *Alc Alc* 1993; 28: 453-459.
20. Guerric C, Montoliu C, Renau-Pigueras J. Involvement of free radical mechanism in the toxic effects of alcohol. Implications for fetal alcohol syndrome. *Adv Exp Med Biol* 1994; 366; 291-305.
21. Fridovich I. Oxygen radicals from acetaldehyde. *Free Radic Biol Med* 1989; 7: 557-558.
22. Nordmann R, Ribiere C, Rouach H. Implication of free radical mechanism in ethanol induced cellular injury. *Free Radic Biol Med* 1992; 12: 219-240.
23. Lucas D, Lamboeuf Y, DeSaint-Blaquet G, Menez JF. Ethanol induced cytochrome P-450 activity and increase in acetaldehyde bound to microsome after chronic administration of acetaldehyde or ethanol. *Alc* 1990; 25: 395-400.
24. Nordey A, Goodnight SH. Dietary lipids and thrombosis, relationships to atherosclerosis: A review. Arteriosclerosis. 1990; 10: 149-163.
25. Jaya DS, Augstin J, Menon VP. Role of lipid peroxides, glutathione and antiperoxidative enzymes in alcohol and drug toxicity. *Ind J Exp Biol* 1993; 31: 453-459.
26. Alexander JC. Chemical and biological properties related to toxicity of heated fats. *J Toxicol Environ Health* 1981; 7: 125-138.

27. Nanji AA, Griniuveim B, Sandrazadeh SM, Levitsky S. Effect of type of fat and ethanol on antioxidant enzyme m-RNA induction in rat liver. *J Lipid Res* 1995; 36: 736-744.
28. Ray G, Hussain SA. Oxidants, antioxidants and carcinogenesis. *Ind J Exp Biol* 2002; 42: 1213-1232.
29. Gerster H. β-carotene, vitamin E and vitamin C in different stages of experimental carcinogenesis. *Eur J Clin Nutr* 1995; 49: 155-168.
30. Dinkova-kostova H, Talalay P. Relation of structure of curcumin analogs to their potencies as inducers of phase-2 detoxification enzymes. *Carcinogenesis*. 1999; 20: 911-914.
31. McClain CJ, Su L Zinc deficiency in the alcoholic: A review. *Clin Exp Res* 1983; 7: 5-10.
32. Fletcher LM. Alcohol and Iron: one glass of red or more? *J Gastroenterol Hepatol* 1996; 11, 1039-1041.
33. Duane P, Raja KB, Simpson RJ, Peters TJ. Intestinal iron absorption in chronic alcoholics. *PMID* 1999; 8985826.
34. Fletcher L, Halliday J, Powell L. Interrelationships of alcohol and iron in liver disease with particular reference to the iron binding proteins, ferritin and transferrin. *J Gastroenterol Hepatol* 1999; 14: 2002.
35. Rodriguez-Moreno F, Gonzalez-Reimers E, Santolaria-Fernandez F, Galindo-Martin Hernandez Torress O, Batista-Lopez N, Molina Perez M. Zinc, copper, manganese and iron in chronic alcoholic liver disease. *Alc* 1997; 14: 39-44.
36. Graf E. Antioxidant potential of ferulic acid. *Free Radic Biol Med* 1992; 13: 435-448.
37. Pannala O, Razaq R, Halliwell B, Singh S, Rice Evans CA. Inhibition of peroxynitrite dependent tyrosine initiation by hydroxy cinnamates: nitration or electron donation. *Free Radic Biol Med* 1998; 24: 594-606.
38. Schroeter H, Williams RJ, Matin R, Iversen L, Rice Evans CA. Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Radic Biol Med* 2000; 29: 1222-1233.
39. Gil M.I, Francisco A, Barberan T, Pierce BH, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem* 2000; 48: 4581-4589.
40. Pan GX, Spencer L, Leary GJ. Reactivity of ferulic acid and its derivative towards hydrogen peroxide and peracetic acid. *J Agric Food Chem* 1999; 47: 3325-3331.
41. Kanski J, Aksenova M, Stoyanova A, Butterfield DA. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure - activity studies. *J Nutr Biochem* 2002; 13, 273-281.

Received: January 19, 2004
Accepted: August 2, 2004

Author's address: Dr. Venugopal P. Menon, Professor and Head, Department of Biochemistry, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India. Phone: 0091-4144-238343, Fax: 0091-4144-238343
E-mail: cmrana@sify.com